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Characterization and multivariate analysis of *Patella intermedia*, *Patella ulyssiponensis* and *Patella vulgata* from Póvoa de Varzim (Northwest Portugal)

Caracterización y análisis multivariante de *Patella intermedia*, *Patella ulyssiponensis* and *Patella vulgata* de Póvoa de Varzim (noroeste de Portugal)

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ABSTRACT

Samples of *Patella intermedia*, *Patella ulyssiponensis* and *Patella vulgata* were collected from the lowest to the highest level of A Ver-o-Mar and Aguçadoura beaches (Póvoa de Varzim, Portugal). Identification of the specimens was based on the morphology of the radula pluricuspid teeth and of the shell. Regressions of shell width or shell apexpos *vs.* shell length yielded slopes not statistically different, but slopes of log shell height *vs.* shell length regression lines were significantly different, indicating that these limpets have different shell growing patterns. However, canonical discriminant analyses using only variables describing shell form yielded poor discrimination between species. Analysis using only variables describing radula relative size improved discrimination, was very satisfactory for *P. ulyssiponensis* specimens, but resulted in a low identification of *P. intermedia*. The best results were achieved using both shell form and radula relative size variables, and the most discriminating variable was radula length/shell height ratio, instead of radula length/shell length as reported in the literature. However, whereas correct identification of *P. ulyssiponensis* specimens was very good (higher than 95 %), for *P. intermedia* and *P. vulgata*, this value ranged from 70 – 80 %. In light of data reported in the literature, morphometric characteristics such as those utilized in this work are probably useful for characterization of *P. ulyssiponensis* as a species, but are of limited value for *P. intermedia* and *P. vulgata*. Qualitative characters are thus still indispensable for the discrimination between these two *Patella* species. Very good qualitative discriminators were, for *P. intermedia*, the tall and broad cusp 2 and the dark marginal rays in the shell interior surface, and for *P. vulgata*, the pointed protuberance on cusp 3, and the silvery head scar.

RESUMEN

Muestras de *Patella intermedia*, *Patella ulyssiponensis* y *Patella vulgata* fueron colectadas en una franja abarcando desde el nivel mas bajo al mas alto de las playas de A Ver-o-Mar y Aguçadoura (Póvoa de Varzim, Portugal). La identificación de los especimenes se basó en la morfología de la concha y de los dientes pluricúspides de la rádula. Las pendientes de las regresiones entre la anchura o el apexpos (distancia entre la proyección del ápice y la parte posterior de la concha) de la concha y la longitud de la concha no difirieron estadís-

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ticamente, mientras que las pendientes de las regresiones entre el logaritmo de la altura y la longitud de la concha fueron significativamente diferentes, indicando que estas lapas presentan patrones de crecimiento distintos. Sin embargo, el análisis canónico discriminatorio utilizando solo las variables que describen la forma de la concha tuvo un bajo poder de discriminación entre especies. Al utilizar solo las variables que describen el tamaño relativo de la rádula se mejoró el poder discriminatorio del análisis, y aunque permitió una identificación satisfactoria de *P. ulyssiponensis*, pero resultó tener bajo poder para la identificación de *P. intermedia*. Los mejores resultados se obtuvieron utilizando variables relacionadas tanto con la forma de la concha como con el tamaño relativo de la rádula, y la variable con mayor poder discriminatorio fue la razón longitud de la rádula/altura de la concha, en lugar de longitud de la rádula/longitud de la concha, como se ha reportado en la bibliografía. Mientras que la correcta identificación de especimenes de *P. ulyssiponensis* fue muy alta (mayor que 95 %), para *P. intermedia* y *P. vulgata*, este valor varió entre 70 – 80 %. A la luz de los datos publicados en la bibliografía, características morfométricas tales como las utilizadas en el presente trabajo, pueden ser de gran utilidad para la caracterización específica de *P. ulyssiponensis*, pero tienen un valor limitado para la caracterización de *P. intermedia* y *P. vulgata*. Las características cualitativas, por tanto, siguen siendo indispensables para la discriminación ente estas dos especies de *Patella*. Para *P. intermedia*, la segunda cúspide alta y ancha, así como los radios marginales oscuros en la superficie interna de la concha, constituyeron muy buenos discriminadores cualitativos, mientras que para *P. vulgata*, lo fueron la protuberancia puntiaguda en la tercera cúspide, junto con la cicatriz de la cabeza de color plateada.

KEY WORDS: *Patella*, radula, canonical discriminant analysis, morphometry.
PALABRAS CLAVE: *Patella*, rádula, anñalisis canónico discriminante, morfometría.

INTRODUCTION

The characterization and delimitation of *Patella intermedia* Murray in Knapp 1857, *Patella ulyssiponensis* Gmelin 1791, and *Patella vulgata* Linné 1758, has been a source of debate and controversy since they were proposed as species (Fisher-Piette, 1935; Rampal, 1965; Ibañez, 1982). This was due to the high shell variability commonly exhibited by these species. In regions of low variability, *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* shells are usually distinct from each other. In regions of high variability, however, some shells display intermediate characteristics, and reliable identification of these species based on shell morphology alone is difficult (Fisher-Piette, 1934, 1948, 1966; Evans, 1947, 1958; Fisher-Piette and Gaillard, 1959). Shell variability of *P. vulgata* and *P. intermedia* is highest in the Basque coast of France and Spain, and in the south limit of their distribution, the Algarve (Portugal) for *P. vulgata*, and northern Africa for *P. intermedia*. *P. ulyssiponensis* is the species with the least variable shell (Fischer-Piette and Gaillard, 1959; Fisher-Piette, 1966).

The difficulties in the identification of *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* specimens based on shell morphology alone led to the search for new characters. Dautzenberg, in the end of the 18th century, pointed out the importance of radula length and pigmentation of mantle tentacle to discriminate several *Patella* species, being therefore the first malacologist to propose alternative characteristics to shell morphology (Fischer-Piette and Gaillard 1959; Rampal, 1965). The work of Fisher-Piette (1934, 1935) showed, for the first time, that the morphology of the radula pluricuspid teeth could be used to characterize several *Patella* species, since they usually display low intraspecific variability, and

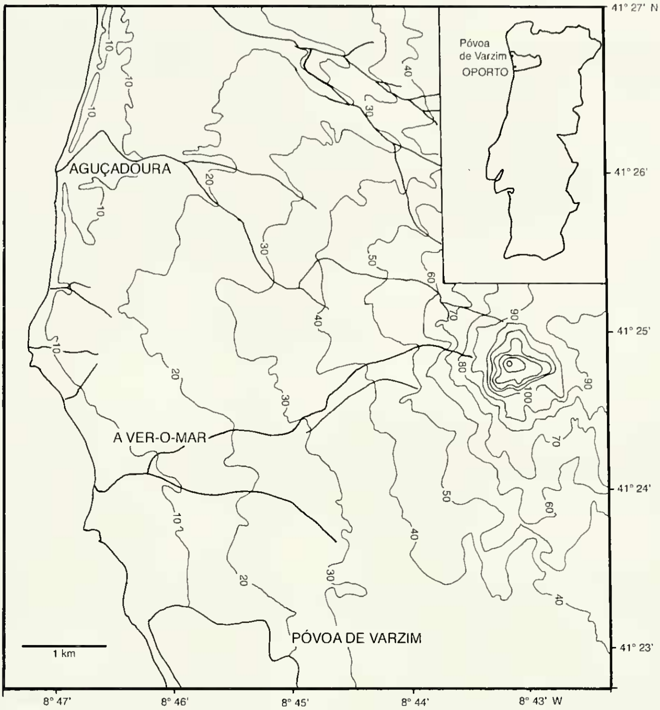

Figure 1. Location of sampling sites (A Ver-o-Mar and Aguçadoura beaches) near Póvoa de Varzim Inset: Localization of the town of Póvoa de Varzim and Oporto district in Portugal.
Figura 1. Ubicación de la zona de muestreo (playas de A Ver-o-Mar y Aguçadoura) en las cercanías de Póvoa de Varzim. Recuadro: Ubicación del pueblo de Póvoa de Varzim y el distrito de Oporto en Portugal.

marked interspecific differences. Fisher-Piette initial observations were confirmed by ESLICK (1940) and EVANS (1947). In a latter paper, FISHER-PIETTE AND GAILLARD (1959) showed that radula unicuspid teeth could also be used to characterize several *Patella* species.

Other characteristics have been proposed for specific discrimination in the genus *Patella*: the foot colour, the breeding seasons, and the ratio of the radula length / cubic root of the shell volume (FISHER-PIETTE, 1935, 1941, 1948; EVANS, 1947, 1953, 1958; FISHER-PIETTE AND GAILLARD, 1959; RAMPAL, 1965; POWELL, 1973).

In this study, samples of *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* from Póvoa de Varzim (northwest Portugal) were examined and compared with respect to several morphological and morphometric characters describing the radula, shell, and soft parts. In particular the following questions were raised:

- How variable are these species in Póvoa de Varzim?

- Is shell form similar in these limpets?

- Are radula and shell characteristics equally important for the separation of these species?

- Can these species be discriminated by morphometric characters alone, rather than by morphological characteristics?

MATERIAL AND METHODS

Collections were made at A Ver-o-Mar and Aguçadoura beaches, two very similar and exposed shores situated

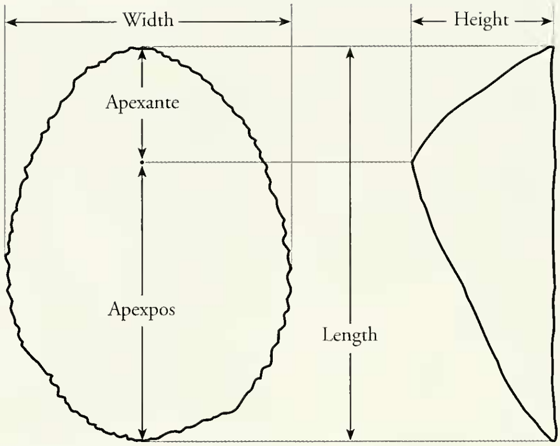

Figure 2. Shell measurements used in canonical discriminant analysis.
Figura 2. Mediciones de la concha utilizadas en el análisis canónico discriminatorio.

near the town of Póvoa de Varzim (Fig. 1). The upper shore is composed of dispersed and heavily eroded granite boulders. Barnacles (*Chthamalus*) cover most of the surfaces and *P. vulgata* is the dominant limpet. Some mussels and gastropods live in the crevices. The middle shore is composed of horizontal granite platforms, very eroded, with small sandy beaches in between. *P. intermedia* and *P. vulgata* are common in middle shore. *P. ulyssiponensis* is restricted to lower shore levels, where *P. intermedia* and *P. vulgata* also occur. Most of the *P. ulyssiponensis* shells are covered with abundant *Gelidium pulchelum* (Turner) Kützing. Common macroalgae in the middle and lower shore include *Bifurcaria bifurcata* R. Ross, *Chondrus crispus* Stackhouse, *Codium tomentosum* (Hudson) Stackhouse, coralline rhodophytes, *Cystoseira* sp., *Enteromorpha* spp., *Gigartina pistillata* (Gmelin) Stackhouse, *Gracilaria verrucosa* (Hudson) Papenfuss, *Lythophyllum* spp., *Mastocarpus stellatus* (Stackhouse in Withering) Guiry and *Sargassum muticum* (Yendo) Feusholt. Sampling took place in January and November 2000, at low tide.

Specimens were collected from the lowest to the highest level of the shores. Squared areas with ca. 30 x 30 cm were marked at random in each level, and all the animals in each area removed from the rock. The total number of collected animals was 608.

Specimens were analysed for foot, radula and shell characteristics. In the laboratory, the animals were observed for the foot colour, and then immersed for a few minutes in boiling water to separate the shell from the soft part. The radula was removed from the visceral mass by dissection, immersed in household bleach to remove mucilaginous substances, washed in distilled water, and measured to the nearest 1mm using a ruler. After air-drying, pluricuspid teeth were first observed using a binocular microscope, with 80 x final magnification. Structural details of the teeth were observed by scanning electron microscopy, in selected specimens. The samples were gold coated (in a JEOL JFC1100 model; film thickness less then 20 nm), and observed in a JEOL JSM-35C model scanning electron microscope, working at 15 keV, with 39 mm working distance.

The external and internal shell surfaces were examined and their characteristics were recorded. Shell length, width, height, apexante and apexpos (Fig. 2 and Table 1 for the definition of these measures) were then determined to the nearest 0.01mm using a digital calliper (Mitutoyo, model CD-15DC).

Table I. Characters used in the canonical discriminant analysis.
Tabla I. Caracteres usados en el análisis canónico discriminante.

Acronym	Description
SL	Shell length: greatest distance between anterior and posterior ends
SW	Shell width: greatest distance perpendicular to the anterior-posterior axis
SH	Shell height: greatest vertical distance from apex of the shell to the plane of aperture
SAA	Shell apexante: greatest distance between apex and anterior end
SAP	Shell apexpos: greatest distance between apex and posterior end
SV	Shell volume = $(\pi/3) \times (SW/2) \times (SL/2) \times SH$
RL	Radula length

Morphometric analysis of the shell and radula was carried out using the variables commonly utilized in studies of patellogastropoda (IBAÑEZ, 1982; IBAÑEZ AND FELIU, 1983; FELIU AND IBAÑEZ, 1984; HERNÁNDEZ-DORTA, 1992; SIMISON AND LINDBERG, 1999). The mean and coefficient of variation of the mean was calculated for each variable and species. Linear regression analysis using the least squares method was applied to pairs of shell or radula variables. These calculations were carried out using Microsoft® Excel 2000 program. Comparison of the slopes was carried out by analysis of covariance using the statistical procedure described by ZAR (1984).

Canonical discriminant analysis on shell and radular morphometric variables was used to reveal differences among the populations, and to identify the variables that were responsible for the majority of separation between species. Discriminant analyses were carried out in several steps. Firstly, using *a posteriori* probabilities and no selection of variables, four discriminant analyses were carried out, two using only shell characters, one with only radula characters, and one using simultaneously shell and radula characters. Secondly, using *a posteriori* probabilities, and simultaneously shell and radula characters, discriminant analysis was carried out with stepwise selection of variables. Significance level "to enter" and "to stay" the variables was set at 0.15. Thirdly, cross-validation analysis (using *a priori* probabilities) was undertaken to assess the reliability of previous findings (using *a posteriori* probabilities). For each species, specimens were re-ordered randomly using Excel 2000 program RAND function. Each group (species) was then divided in two subgroups. For *P. intermedia*, these contained 152 specimens each, for *P. ulyssiponensis*, 33 and 32, each, and for *P. vulgata*, 119 and 120. The first group (the analysis sample containing the training or calibration data) was used to derive the discriminant functions, coefficients, and loadings. The second group (the houldout sample containing the test data) was used to test the discriminant functions, by classifying each specimen into one of the *Patella* species. This process was repeated ten times. At each run, a percent correct identification to species was evaluated. The means of these values were compared with those obtained by using *a posteriori* probabilities. Discriminant analyses were performed using the NCSS (NCSS Statistical Software, Utah, USA) and XLSTAT 5.1 (Addinsoft, Paris, France) software packages.

RESULTS

I. Delimitation of species

Based on radular and shell morphology, the collected specimens were divided into three groups. Within each group, radula pluricuspid teeth displayed low variability, and shells exhibited limited morphological variation.

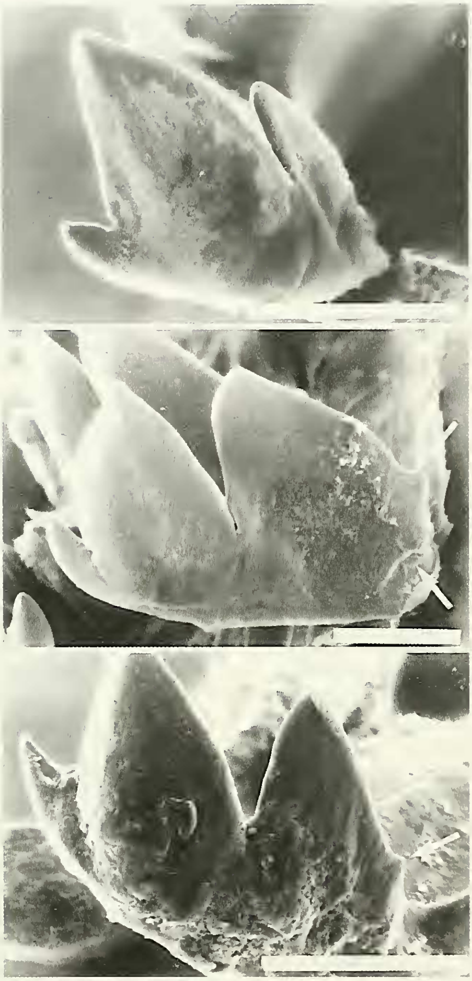

Figure 3. Morphology of pluricuspid teeth of the radula. Numbering of the cusps begins from left to the right (corresponding to the outer and inner sides of the radula). Top: *Patella intermedia*. Cusp 2 is taller and broader than cusps 1 and 3. Middle: *Patella ulyssiponensis*. Cusp 2 is directed to cusp 3. Cusp 3 is wither than cusp 2. Cusp 3 has a protuberance on its outer side. Bottom: *Patella vulgata*. Cusps 2 and 3 subequal. Cusp 3 has a pointed projection on its outer side border. Scale bars 100 µm.
Figura 3. Morfología de los dientes pluricúspides de la rádula. La numeración de las cúspides comienza desde la izquierda hacia la derecha (correspondiendo a los lados externos e internos de la rádula). Superior: Patella intermedia. *La segunda cúspide es más alta y ancha que la primera y tercera. Medio:* Patella ulyssiponensis. *La segunda cúspide está dirigida hacia la tercera. Esta última es más ancha que la segunda. La tercera cúspide tiene una protuberancia sobre el lado externo. Inferior:* Patella vulgata. *Las segunda y tercera cúspides son subiguales. La tercera tiene una proyección puntiaguda en el borde externo. Escalas 100 µm.*

The three groups displayed the following features:

Group I. Three unequal pluricuspid teeth (Figure 3, top). Cusp 2 (centre) much taller and broader than cusps 1 (left) and 3 (right). Margin of the shell rimose, with pointed extensions connected to the rays (Figure 4). External surface with few and prominent ribs. Interior with alternating dark and light

Figure 4. *Patella intermedia* shells from A Ver-o-Mar and Aguçadoura beaches.
Figura 4. Conchas de Patella intermedia *provenientes de las playas de A Ver-o-Mar y Aguçadoura.*

rays, in the lower part. Head scar yellow-orange, but creamy in a few specimens.

Group II. Three unequal pluricuspid teeth (Figure 3, middle). Cusp 1 (left) very small. Cusp 2 (centre) bent to cusp 3 (right). Base of cusp 3 wider than cusp 2. Cusp 3 with a protuberance on its outer side – a vestigial fourth cusp. Margin of the shell finely crenulate (Figure 5). External surface crowded by numerous, closely spaced, well-marked ribs, of unequal size. Interior homogeneous, porcelanous white. Head scar white, creamy or pale orange.

Group III. Three unequal pluricuspid teeth (Figure 3, bottom). Cusp 1 (left) small. Cusps 2 (centre) and 3 (right) subequal. Cusp 3 with a pointed protuberance on its outer side border. Margin of the shell entire or slightly indented (Figure 6). External surface smooth at the apex, with flat and spaced ribs below. Interior with transparent nacre, often with a green or blue iridescence. Silvery head scar.

Based on shell and radula morphological characters reported in the literature for *Patella* spp. (Fisher-Piette, 1934, 1935; Fisher-Piette and Gaillard, 1959; Evans, 1947, 1953; Rolán, 1993; Rolán and Otero-Schmitt, 1996), groups I, II and III were unequivocally identified as belonging to *P. intermedia*, *P. ulyssiponensis* and *P. vulgata*, respectively.

Radula pluricuspid teeth of *P. intermedia* from Póvoa de Varzim is similar to *P. intermedia* type B of Evans (1953), and to those reported by Fisher-Piette (1934, figure 2), Fisher-Piette (1935, figure 9, figure 17-2) and Fisher-Piette and Gaillard (1959, figure 11 A and B).

Radula pluricuspid teeth of *P. ulyssiponensis* from Póvoa de Varzim is similar to *P. ulyssiponensis* type A of Evans (1953), and to those reported by Fisher-Piette (1934, figure 2), Fisher-Piette (1935 – figure 10, figure 17-9) and Fisher-Piette and Gaillard (1959 – figure 14 B).

Radula pluricuspid teeth of *P. vulgata* from Póvoa de Varzim is similar to *P. vulgata* types A and B of Evans (1953), and to those reported by Fisher-Piette (1934, figure 2), Fisher-Piette (1935, figure 8, figure 17-1) and Fisher-Piette and Gaillard (1959, figure 9A).

P. intermedia, *P. ulyssiponensis* and *P. vulgata* shells from Póvoa de Varzim are morphologically similar to those found in Galiza (northwest Spain) (Rolán, 1993; Rolán and Otero-Schmitt, 1996) and at Cardigan Bay (Wales, Great Britain; Evans, 1947).

The foot showed no constant colouration within each group, and therefore was not used in the identification. *P. intermedia* foot was dark, from grey or yellow to black. *P. ulyssiponensis* foot was light, yellow or, most often, orange. The colour of *P. vulgata* foot was very variable, from light yellow to black.

II. Morphometric delineation of species

For all three *Patella* species, SW and SA was a linear function of SL (Table II). However, SH was a curvilinear function of SL, indicating that as these limpets increase in size, the relative height of the shell increases by a power function. Similar relationship between SH and SL has been reported for several *Notoacmea* (Lindberg, 1982) and *Patella* species (Davies, 1969; Muñoz and Acuña, 1994). Correlation between SH and SL increased after log transformation of SH, indicating that an exponential fit was a better approach than a linear function.

Regressions of SW or SAA *vs.* SL for *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* specimens yielded slopes not statistically different (Table II). However, slopes of log SH *vs.* SL regression lines were significantly different (Table II), indicating that the relative increase in height as the shell grows was not uniform in these *Patella* species. Slopes increased in the order *P. ulyssiponensis*, *P. vulgata*, and *P. intermedia*.

For all three *Patella* species, RL was a linear function of SL and of $\sqrt[3]{(SV)}$ (Table II). Similar relationship between RL and SL has been reported for several *Patella* species (Brian and Owen, 1952; Davies, 1969; Sella, 1976). Slopes of the regression lines were significantly different, and increased in the order *P. ulyssiponensis*, *P. intermedia*, and *P. vulgata* (Table II).

In *P. intermedia*, *P. ulyssiponensis* and *P. vulgata*, radula relative size displayed higher variability than shell form. Whilst the coefficient of determination for the regressions describing shell form (SW *vs.* SL, log SH *vs.* SL, and SAP *vs.* SL) ranged between 0.597 and 0.952, those describing radula relative size (RL *vs.* SL, and RL *vs.* $\sqrt[3]{(SV)}$) varied between 0.472 and 0.767 (Table II). Whilst the coefficient of variation of the means describing shell form (SW/SL, SH/SL, SAA/SAP, and SAP/SL) varied between 5.4 and 22 %, those describing radula relative size (RL/SL, RL/SH,

Figures 5, 6. *Patella* shells from A Ver-o-Mar and Aguçadoura beaches. 5: *P. ulyssiponensis*; 6: *P. vulgata*.
Figuras 5, 6. Conchas de Patella *provenientes de las playas de A Ver-o-Mar y Aguçadoura. 5:* P. ulyssiponensis*; 6:* P. vulgata.

Table II. Slopes of regression lines for morphometric characterization of the species, and their statistical comparison.
Tabla II. Pendientes de las rectas de regresión de la caracterización morfomética de las especies, y su comparación estadística.

Regression	*P. intermedia*		*P. ulyssiponensis*		*P. vulgata*		Comparison of slopes (DF=2, 604)
	Slope	r^2	Slope	r^2	Slope	r^2	F
Shell							
SW *vs.* SL	0.859	0.952	0.791	0.918	0.821	0.929	3.719^{NS}
Log_{10} SH *vs.* SL	0.0241	0.744	0.0149	0.597	0.0182	0.754	23.1***
SAP *vs.* SL	0.608	0.859	0.570	0.834	0.627	0.939	2.311^{NS}
Radula							
RL *vs.* SL	1.29	0.472	0.712	0.625	1.40	0.501	8.557***
RL *vs.* $\sqrt[3]{(SV)}$	2.89	0.485	1.89	0.767	3.15	0.556	5.690***

^{NS} Not significant at 0.02 level
*** Significant at 0.005 level

RL/SAA, and RL/$\sqrt[3]{(SV)}$) varied 9.6 and 28 % (Table III). Concerning the radula relative size, *P. ulyssiponensis* was the least variable species (Table III).

III. Discrimination between species

In order to determine which variables provided the best discrimination between species, several canonical discriminant analyses were carried out using different techniques and different sets of radula and/or shell characteristics. Results are displayed in Tables IV - VII.

Analysis I and II, using only variables describing shell form, resulted in a very poor discrimination between species, with only 65 % of the specimens *a posteriori* correctly identified to species (Table IV). The first canonical variable accounted for 91 % of the variation between species, and the variable with the highest loading was SH/SL (data not shown). Analysis III, using only variables describing radula relative size, was better than analyses I and II, and was very satisfactory for *P. ulyssiponensis*, but resulted in a low *a posteriori* correct identification of *P. intermedia* specimens (Table IV). The first canonical variable accounted for 76 % of the variation between species, and the variables with the highest loadings were RL/SH followed by RL/$\sqrt[3]{(SV)}$ (data not shown) The simultaneous use of variables describing shell form and variables describing radula relative size (Analysis IV) was better than the use of one of these groups of variables alone (Table IV). However, the improvement of Analysis IV over Analysis III was limited. *A posteriori* correct identification of *P. ulyssiponensis* specimens was unchanged (and very good), but identification of *P. intermedia* and *P. vulgata* specimens was lower than 80 % (Table IV). The first canonical variable accounted for the great majority of the variation between species, and the second canonical discriminant variable accounted for only 23.7 % of the variance (Table VI). The variables that provided the highest contrast between the three *Patella* species were RL/SH followed by RL/$\sqrt[3]{(SV)}$, and then RL/SL (Table VII). Individuals were plotted along the two canonical variables (Figure 7). There was a considerable overlap between *P. intermedia* and *P. vulgata* specimens, but a reasonable discrimination of *P. ulyssiponensis* individuals. Stepwise selection of variables describing shell form and radula rela-

Table III. Morphometric characterization of the species.
Tabla III. Caracterización morfométrica de las especies.

Variable	*P. intermedia* (n= 304)		*P. ulyssiponensis* (n= 65)		*P. vulgata* (n= 239)	
	Mean	CV (%)	Mean	CV(%)	Mean	CV(%)
Shell						
SW/SL	0.822	5.8	0.764	6.3	0.793	5.6
SH/SL	0.279	19	0.318	18	0.346	17
SAA/SAP	0.479	22	0.509	16	0.523	15
SAP/SL	0.682	7.0	0.665	10	0.658	5.4
Radula						
RL/SL	1.68	19	0.934	12.4	1.55	20
RL/SH	6.24	26	2.99	14	4.54	20
RL/SAA	5.44	28	2.84	22	4.59	25
RL/$\sqrt[3]{(SV)}$	4.32	19	2.35	9.6	3.73	19

Table IV. Number and percent correct identification of the specimens, based on canonical analyses using different sets of shell and/or radula characters.
Tabla IV. Número y porcentaje de identificación correcta de los especímenes, basado en el análisis canónico usando diferentes conjuntos de caracteres de concha y/o rádula.

Analysis	Variables	*P. intermedia* (n= 304)		*P. ulyssiponensis* (n= 65)		*P. vulgata* (n= 239)		Overall Mean
		N	%	N	%	N	%	%
I	SW/SL SH/SL SAA/SAP	223	73.4	34	52.3	138	57.7	65.0
II	SW/SL SH/SL SAP/SL	222	73.0	34	52.3	139	58.2	65.0
III	RL/SL RL/SH RL/SAA RL/$\sqrt[3]{(SV)}$	202	66.4	64	98.5	194	81.2	75.7
IV	SH/SL SAA/SAP RL/SL RL/SH RL/SAA RL/$\sqrt[3]{(SV)}$	220	72.4	64	98.5	187	78.2	77.5
V	SH/SL RL/SL RL/SH RL/SAA RL/$\sqrt[3]{(SV)}$	222	73.0	64	98.5	187	78.2	77.8

Table V. Percent correct identification of the specimens based on canonical discriminant analysis using shell and radula characters (SH/SL, SAA/SAP, RL/SL, RL/SH, RL/SAA, and RL/$^3\sqrt{}$(SV)). Cross-validation analysis using half number of specimens for calibration and half for test.
Tabla V. Porcentaje de identificación correcta de los especímenes, basada en el análisis canónico discriminante usando caracteres de la rádula y concha (SH/SL, SAA/SAP, RL/SL, RL/SH, RL/SAA, and RL/$^3\sqrt{}$(SV)). Análisis de validación cruzada usando la mitad de los especímenes para la calibración y la otra mitad para el test.

	P. intermedia	*P. ulyssiponensis*	*P. vulgata*	Overall Mean
Mean	70.9	96.6	77.1	76.0
CV (%)	5.6	3.6	5.2	3.3

Table VI. Eigenvalues and Wilks' λ of canonical discriminant analysis using shell and radula characters (SH/SL, SAA/SAP, RL/SL, RL/SH, RL/SAA, RL/$^3\sqrt{}$(SV)). Significance of Wilks' λ was evaluated by Fisher's F values.
Tabla VI. Autovalores y λ de Wilks del análisis canónico discriminante usando caracteres de la concha y rádula (SH/SL, SAA/SAP, RL/SL, RL/SH, RL/SAA, RL/$^3\sqrt{}$(SV)). La significatividad de la λ de Wilks se evaluó con los valores de la F de Fisher.

	Eigenvalue	% of Variance Individual	% of Variance Cumulative	Canonical correlation	Wilks' λ
First canonical variate	0.820	76.3	76.3	0.671	0.438***
Second canonical variate	0.255	23.7	100	0.450	0.797***

*** Significant at 0.01 level

Table VII. Total canonical structure for discriminant analysis of *Patella* species. Variables with the highest loadings are in bold.
Tabla VII. Estructura canónica total para el análisis discriminante de las especies de Patella. *Las variables con mayores cargas están en negrita.*

Variable	First canonical variable	Second canonical variable
SH/SL	-0.556	0.711
SAA/SAP	-0.306	0.327
RL/SL	0.777	**0.607**
RL/SH	**0.969**	-0.053
RL/SA	0.758	0.274
RL/$^3\sqrt{}$(SV)	**0.901**	0.388

tive size (Analysis V, Table IV) resulted in the removal of SAA/SAP, but the *a posteriori* correct identification to species was only very slightly improved. The removal of SAA/SAP was expected since it was the variable that provided the lowest contrast between the three limpet species (Table VII).

Analysis I – V were carried out using *a posteriori* probabilities. In this technique, the same data set that is used to derive the discriminant functions is also

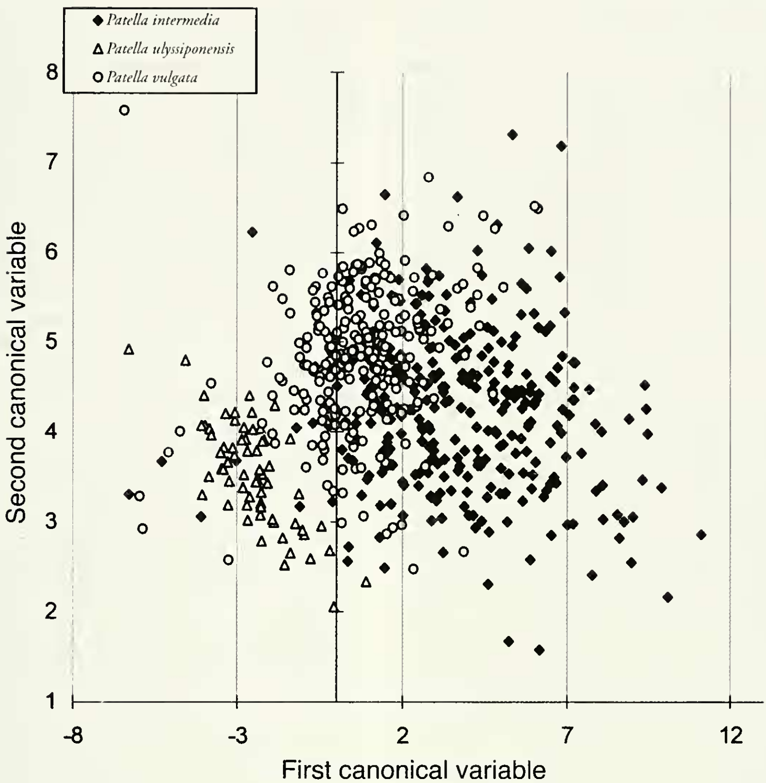

Figure 7. Scatter plot of the first and second canonical variables in the discriminant analysis using shell and radula measurements (Analysis IV). The variables most closely associated with the discrimination between species were RL/SH and RL/$\sqrt[3]{(SV)}$.

Fig. 7. Gráfica de la primera y segunda variables canónicas del análisis discriminatorio realizado utilizando mediciones de la concha y la rádula (Análisis IV). Las variables que más fuertemente influencian la discriminación entre especies fueron RL/SH y RL/$\sqrt[3]{(SV)}$*.*

used to test the accuracy of the predictions using these functions. In order to test the reliability of these results, an *a priori* technique was used with a cross-validation of the data. Mean percentages of correct identification of the specimens using this technique (Table V) were very similar to those obtained with *a posteriori* probabilities (Table IV, Analysis IV), indicating high within-group (species) homogeneity of specimens. Only *P. ulyssiponensis* specimens were very well correctly identified. Percent correct identification of *P. intermedia* and *P. vulgata* specimens were again lower than 80 % (Table V).

DISCUSSION

I. Shell form

Results presented in this work showed that *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* from Póvoa de Varzim region have different shell growing patterns. Although SW or SAA *vs.* SL regressions showed slopes not significantly different in these *Patella* species, log SH *vs.* SL regressions displayed significantly different slopes. The fastest growing species in height was *P. intermedia*, and the slowest *P. ulyssiponensis*, *P. vulgata* displaying intermediate behaviour.

II. Radula relative size

Mean RL/SL ratios for *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* reported in the literature for several coastal regions of Spain, France, Great Britain and Ireland display the following ranges: 1.60-2.20, 0.94-1.20, 1.29-2.29, respectively (FISHER-PIETTE, 1934, 1935, 1941, 1948, EVANS, 1947, 1953, 1958; BRIAN AND OWEN, 1952; FISHER-PIETTE AND GAILLARD, 1959; CHRISTIAENS, 1973; IBAÑEZ, 1982; FELIU AND IBAÑEZ, 1984). Mean RL/$\sqrt[3]{(SV)}$ ratios for *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* in several coastal regions of Spain, France, and Great Britain show the following variation: 4.66-4.71, 2.87-2.90, 3.90-4.38, respectively (Fisher-Piette, 1941, 1948). Our results for Póvoa de Varzim (northwest Portugal) (Table III) were within, or very near these ranges.

P. ulyssiponensis is usually the least variable of these three limpets, if we consider the inter-site and within-site variability of the radula relative size (FISHER-PIETTE, 1934, 1935, 1941, 1948, EVANS, 1953, 1958; IBAÑEZ, 1982; FELIU AND IBAÑEZ, 1984). This conclusion is illustrated by data reported by IBAÑEZ (1982), IBAÑEZ AND FELIU (1983) and FELIU AND IBAÑEZ (1984) on several *Patella* species in the Basque coast. RL/SL ratios were determined at several sites in this region, and on different months and different levels, at each site. *P. ulyssiponensis* displayed similar RL/SL ratios along the year and between different levels in the beach. On the contrary, RL/SL ratios for *P. intermedia* and *P. vulgata* varied appreciably along the year, between sites and between different levels at each site. Generally, RL/SL ratios increased from winter to summer months. Our results for these species in Póvoa de Varzim agree with this conclusion. The coefficient of variation for the means of all variables describing the radula relative size were lowest in *P. ulyssiponensis*.

Data reported in the literature indicate that in the genus *Patella*, the radula relative size decreases, with lowering the level of the occurrence of the animal in the shore. This general trend has been observed both within each species, and between several species. BRIAN AND OWEN (1952) studied the variability of the RL/SL ratio for *P. vulgata* in the west coast of Great Britain. Whilst animals from high-water habitats, above high-water neaps, displayed RL/SL ratios in the range 1.83-1.96, animals from low-water habitats, about or below low-water neaps, showed RL/SL ratios between 1.38-1.61. DAVIES (1969) observed that in two sites in the Italian coast, intertidal populations of *Patella caerulea* Linné 1758 displayed longer radula than submerged populations. SELLA (1976) studied the variability of the RL/SL and RL/SH ratios in *Patella aspera* Röding 1798 (synonym *P. ulyssiponensis*) and *P. caerulea* from several Mediterranean stations in the Tyrrhenian Sea. In both species, both ratios decreased from animals living in the mesolittoral zone (50 cm above to 50 cm below water level) to animals living in the infralittoral region (6 to 26 m depth). In *P. ulyssiponensis*, the RL/SL ratio decreased, from the mesolittoral to the infralittoral zones, from 0.80 to 0.66 at one station, and from 0.90 to 0.64 at another site. The RL/SH ratio decreased from 3.2 to 3.0 and from 3.4 to 3.0, at the same sites and depths, respectively. Our observations that radula relative size was more variable than shell form, could have been due to this dependence of the radula relative size on the position of the animal in relation to the water level, since the animals were collected from the low shore to high shore levels, and all specimens of each species analysed as a unique set. However, the coefficient of variation of mean RL/SL and RL/$\sqrt[3]{(SV)}$ ratios was at maximum 20 %, a measure of variability not uncommonly found in biological populations. The exact extent of the variability of the radula relative size is expected to vary between sites in the European coasts, considering the polymorphism of the shorelines and the variability of tides in this area.

IBAÑEZ (1982) and IBAÑEZ AND FELIU (1983) found that in the Basque Coast, *Patella rustica* Linné 1758, *P. vulgata*, *Patella depressa* Pennant 1777 (synonym *P. inter-*

media) and *P. aspera* (*P. ulyssiponensis*) occupy successively lower positions in the coast. Mean RL/SL ratios for these species were 3.32, 1.76, 1.60-2.18, 0.986, respectively. HERNÁNDEZ-DORTA (1992) reported that in the Canary Islands, *Patella candei* d'Orbigny 1840, *Patella piperata* Gould 1846, *P. ulyssiponensis* and *Patella crenata* Gmelin 1791 (synonym *P. ulyssiponensis*) habitats decreased in height in the shoreline. Mean RL/SL ratios found for these species in the region were 1.63, 2.29, 0.74 and 1.12, respectively. This general trend of decreasing radula size from mesolittoral to the infralittoral species has also been found at Póvoa de Varzim. *P. ulyssiponensis* occupies the lowest positions in the shore, and displayed the lowest RL/SL and RL/$^3\sqrt{}$(SV) ratios.

III. Discrimination between species

Our results presented in this work showed that, in the discrimination between *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* from Póvoa de Varzim, variables describing the radula relative size were more important than variables describing shell form. Whilst using only variables related to shell form, the mean *a posteriori* correct identification to species was 65.0 %, using only variables describing the radula relative size this value rose to 75.7 %. Our results therefore confirm the observations reported by several malacologists (see Introduction) on the importance of the characteristics of the radula to the discrimination between *P. intermedia*, *P. ulyssiponensis* and *P. vulgata*.

The RL/SL ratio is considered the most important morphometric variable for discriminating European *Patella* species. HERNÁNDEZ-DORTA (1992) comparing *P. candei*, *P. piperata* and *P. ulyssiponensis* from the Canary Islands concluded that, from several shell and radular variables, RL/SL was the most discriminating variable, followed by SH/SL. However, in the present work, for the discrimination between *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* from Póvoa de Varzim, the most important variable was RL/SH, both in the analysis using only radula relative size variables and in the analysis using both shell form and radula size variables. RL/$^3\sqrt{}$(SV) was the variable with the second highest loading, and RL/SL the third. This is a new finding, and can be due to the use, in the present analysis, of a wider range of variables describing the radula relative size, four instead of one or two commonly used. Alternatively, our results can be related to a specificity of these species at this site.

This observation that RL/SH was the most discriminating variable between *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* confirms other results also found in this study on the importance of the SH in the discrimination between these species. Using only variables describing shell form, SH/SL was the most discriminating variable. Whilst slopes of the regressions, SW or SAA *vs.* SL were not statistically different, slopes of log SH *vs.* SL regression lines were significantly different.

The best discrimination between *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* from Póvoa de Varzim was achieved by using shell form and radula relative size variables. However, the overall mean correct identification to species (using *a priori* or *a posteriori* probabilities) was below 80 %, and only *P. ulyssiponensis* specimens were correctly identified in high percentage (higher than 95 %). It appears therefore that quantitative characters related to shell form and radula size are relatively poor discriminators between *P. intermedia* and *P. vulgata*. Qualitative characters are thus still indispensable for the discrimination between these two *Patella* species. Very good qualitative discriminators were, for *P. intermedia*, the tall and broad cusp 2 and the dark marginal rays in the shell interior surface, and for *P. vulgata*, the pointed protuberance on cusp 3, and the silvery head scar.

IV. The use of Canonical Discriminant Analysis in the discrimination between *Patella* species

Canonical discriminant analysis has been used in the literature to compare limpet species. These analyses have used variables describing the shell and the radula - shell size and radula *absolute* size variables alone, or simultaneously, with shell form and radula relative size variables, which were the variables exclusively used in the present work. The use of variables describing shell and radula *absolute* sizes in the comparison between limpet species has however some limitations, because limpets present extremely variable populations structure. LEWIS AND BOWMAN (1975) and THOMPSON (1980) reported detailed studies on the biology and population dynamics of *P. vulgata* in several sites of the England and Ireland coasts. Very marked differences in length-frequency distributions were observed between populations occupying different tidal levels and different habitats. GUERRA AND GAUDENCIO (1986) studied several populations of *P. aspera* (*P. ulyssiponensis*), *P. depressa* (*P. intermedia*) and *P. vulgata* from the Portuguese coast, with monthly sampling. All these three species exhibited marked variations in the population structure throughout the year, and at a given month, these limpets showed different populations structures. These differences in populations structures have been interpreted as the result of a complex set of factors - gonad cycles, spawning and settlement periods, growth rates, survival, mortality and longevity, or in brief, as the result of population history (LEWIS AND BOWMAN, 1975; THOMPSON, 1980; GUERRA AND GAUDENCIO, 1986). Therefore, unless the populations that are compared have similar structures, namely percentage of juveniles and adults, and sampling is exhaustive and based on representatives of all age-classes, comparisons between variables describing shell and radula *absolute* sizes might not represent real differences between species.

CONCLUSIONS

P. intermedia, *P. ulyssiponensis* and *P. vulgata* from Póvoa de Varzim region have different shell growing patterns.

The best discrimination between *P. intermedia*, *P. ulyssiponensis* and *P. vulgata* was achieved by using shell form and radula relative size variables. However, whereas correct identification of *P. ulyssiponensis* specimens was higher than 95 %, the identification of *P. intermedia* and *P. vulgata* specimens ranged from 70 to 80 %. Qualitative characters describing the radula and the shell are thus still indispensable for the discrimination between these two limpet species.

Morphometric characteristics such as those utilized in this work are probably useful for characterization of *P. ulyssiponensis* as a species, but are of limited value for *P. intermedia* and *P. vulgata*.

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